Quaternary amines affect the pupil size comparable to their tertiary amine counterparts

Quaternary, tertiary amines and the pupil

Shahnawaz Channa[1], Elhassan Hussein Eltom[2], Mohammad Akram Randhawa[3]
[1]Department of Ophthalmology, Northern Border University, Arar, Saudi Arabia
[2]Department of Pharmacology, Northern Border University, Arar, Saudi Arabia
[3]Department of Pharmacology, Hazrat Bari Sarkar Medical & Dental College, Islamabad, Pakistan

Abstract

Aim: In this study, we aimed to investigate and compare the effect of topical administration of quaternary amines (QA) to tertiary amines (TA) on the pupil size of rabbit eyes.

Material and Methods: TA (Pilocarpine and Physostigmine) and QA (Neostigmine and Pyridostigmine) muscarinic agonists were used as pupil constrictors, while TA (Tropicamide and Cyclopentolate) and QA (Hyoscine-N-butylbromide, Ipratropium, Isopropamide, and Hexahydro-adephenine) muscarinic antagonists were used as pupil dilators. Serial dilutions of these drugs (4 concentrations for each) were prepared in distilled water from their preparations available on the market. Their effect on pupil size was measured at 0, 15, 30, 45, 60, 120, and 180 minutes (n=4). Linear plots of the effects of various concentrations were drawn by MS-Excell. The onset and duration of action of QA were compared with the corresponding TA.

Results: The onset of action of pupil constriction with TA and QA muscarinic agonists was between 15-30 minutes and the effect lasted for 120-180 minutes. The onset of action of pupil dilatation with TA and QA was also between 15-30 min and effect lasted for > 180 minutes.

Discussion: This experimental study demonstrated that the extent of ionization of QA muscarinic agonists or antagonists did not influence their diffusion across ocular tissues cornea and conjunctiva. These drugs can be used topically for pupillary constriction or dilatation.

Keywords

Quaternary amines; Tertiary amines; Pupil size

Introduction

A large number of drugs are weak bases and most of these bases are amines. These amines may be primary, secondary, or tertiary according to the groups associated with the nitrogen of a neutral amine. A primary amine has one carbon (R) and two hydrogens (H), a secondary amine has two carbons and one hydrogen, and a tertiary amine has three carbon atoms attached to the nitrogen of amine (Figure 1: a, b, c). Each of these three forms may reversibly bind a proton with the unshared electron. A quaternary amine has four carbon atoms (Figure 1: d), is permanently charged, and has no unshared electron with which a proton could bind reversibly.
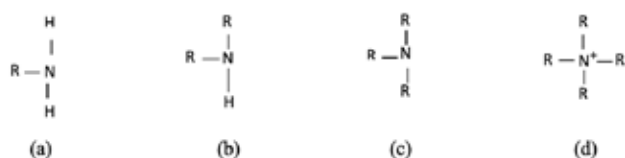
**Figure 1.** Structure of primary (a), secondary (b), tertiary (c), and quaternary amines (d) [1]

The primary, secondary, and tertiary amines (TAs) may undergo reversible ionization and vary their lipid solubility with pH, but quaternary amines (QA) are always in the poorly lipid-soluble charged state [1]. Therefore, it is predicted that the absorption of QA in the tissues is poor, as compared to TAs. TAs are well absorbed in all tissues and can be used topically in the eye [2]. Similarly, it was mentioned that pilocarpine and arecoline, being tertiary amines, can readily be absorb from all sites and can readily cross the blood-brain barrier and also affect central nervous system. However, muscarine, which is a quaternary amine, can also produce effects on central nervous system when ingested [3].

Weakly basic or weakly acidic drugs are present in the solution in unionized and ionized form. The unionized form is considered more lipid-soluble and easily diffusible across the body membranes, whereas, the ionized form is lipid-insoluble and difficult to cross the body membranes.

The ratio of unionized to ionized (U/I) forms of a drug at a particular pH can easily be calculated using the Henderson-Hasselbalch equation (H-H equation) when its pKa (pH at which 50% of the drug is ionized) is known. The H-H equation relates the ratio of ionized to unionized weak base or weak acid to the drug's pKa and the pH of the medium as shown below [1,4]:

$$\text{Log [(ionized) / (unionized)]} = pKa + pH$$

It is evident from the equation that weakly acidic or weakly basic drugs with the similar pKa at the same pH of the medium should have an equal U/I ratio and the similar membrane transport capability. But, the U/I ratio at pH4 for 7 acidic drugs with similar pKa values (from 3.5 to 5.6) had widely different capacities for crossing buccal mucosa at pH4. The correlation coefficient (R2) between the U/I ratio (calculated using H-H equation) at pH4 and the percentage of buccal absorption (BA) determined by the Beckett and Triggs method at pH4 was
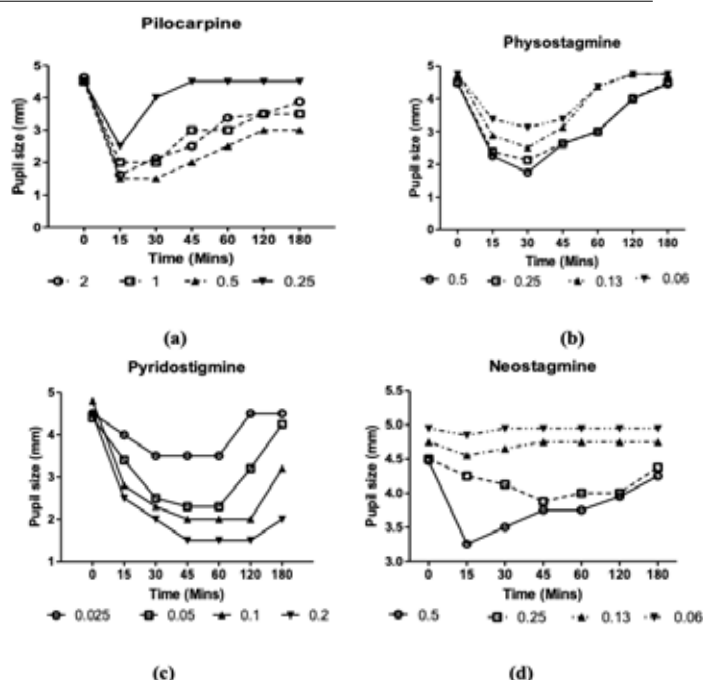
Figure 2. Linear plot of mean (n = 4) pupil size (mm), Y-axis, versus time (minutes), X-axis, for different concentrations of TA muscarinic agonist (pupil constrictors).
(a) Pilocarpine (b) Physostigmine (c) Neostigmine and (d) Pyridostigmine
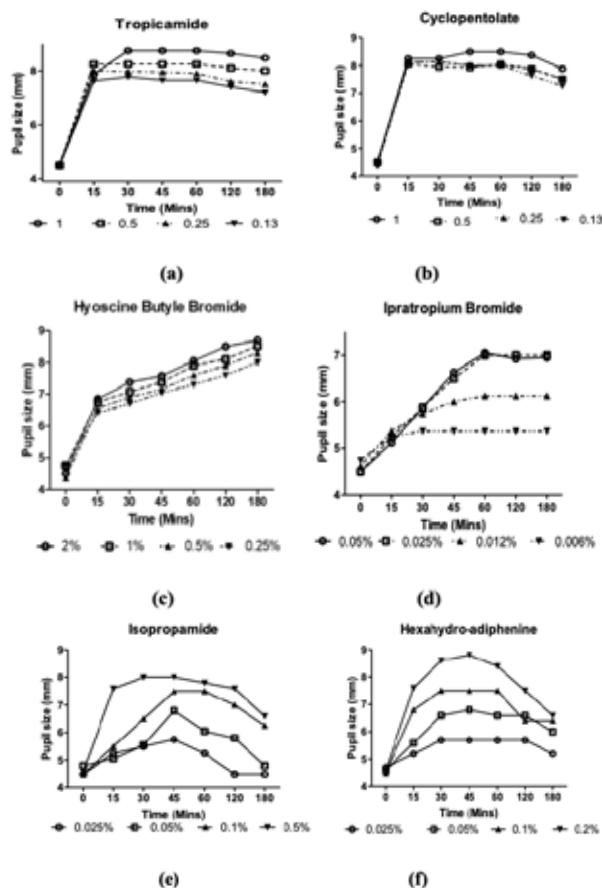
Figure 3. Linear plot of mean (n = 4) pupil size (mm), Y-axis, versus time (minutes), X-axis, for different concentrations of TA muscarinic antagonists (pupil dilators) (a)Tropicamide (b) Cyclopentolate (c) Hyoscine Butyl Bromide (d) Ipratropium Bromide (e) Isopropamide and (f) Hexahydro-adiphenine

0.0527 [5-6]. Similarly, the U/I ratio at pH9 (calculated using H-H equation) for 10 basic drugs with similar pKa values (9 to 9.6) had widely different capacities to cross buccal mucosa and the R2 between their U/I ratio at pH9 and BA percentage at pH 9 was 0.0583 [7]. Moreover, 10 basic drugs with similar pKa (9 to 9.6) had widely different profiles for pH-dependent renal excretion and the R2 for the correlation between their pKa and acid/alkaline (Ac/Al) ratio for 24-hour urine excretion was 0.0192 [6].

However, it is reported that ionization calculated according to the H-H equation had a poor correlation with a membrane transport capacity of drugs, and therefore the equation is considered inadequate for determining the diffusion of drugs across the body membranes [7]. It is suggested that the ionization of QA should also not affect their permeability through the ocular tissues like conjunctiva and cornea. Thus, the QA should be able to constrict or dilate the pupil.

Therefore, this experimental study was conducted to compare QA, muscarinic-agonists, and antimuscarinic antagonists (pupil constrictors and dilators, respectively) to TA (pupil constrictors and dilators) in terms of their effect on the pupil size of the rabbit eyes after topical administration.

Material and Methods

Preliminary ethical approval was obtained from the Deanship of Scientific Research for this study under project No. 7526-MED-2017-1-8-F.

Pilocarpine and physostigmine from TA and neostigmine and pyridostigmine from QA were used as pupil constrictors. Tropicamide and cyclopentolate from TA and Hyoscine butyl bromide, Ipratropium, Isopropamide, and Hexahydro-adiphenine from QA were used as pupil dilators.

Different concentrations of drugs, for the already available as eye drops, were prepared in distilled water. For the drugs which were not available as eye drops, concentrations were prepared from their base powders or injections obtained from the market. Four adult rabbits, weighing 2-3 kg were used in the current study. Rabbits were randomly selected for each concentration of the drug. Two drops of the drug were instilled in the right eye (test) and only normal saline in the left (control) at 9 a.m. The pupil diameter of both eyes with the same amount of diffused light was measured with the help of pupil-meter (Alcon Laboratories; USA) at 0, 15, 30, 60, 120, and 180 minutes. The procedure was done for four different concentrations of each drug in all the 4 animals.

To demonstrate the onset of action and the duration of the effect, linear graphs of the mean (n = 4) pupil size (mm) versus time (minutes) with different concentrations of TA and QA (for both the pupil constrictor and the pupil dilators) were made with the help of Microsoft EXCEL.

Results

For the pupil constrictors, the mean pupil size (± SD, n=4) of TA (Pilocarpine and Physostigmine) and QA (Neostigmine and Pyridostigmine) was measured at 0, 15, 30, 60, 120 and 180 minutes for different concentrations of each drug (Table 1). The onset of action of pupil constriction with TA (Pilocarpine and Physostigmine) and QA (Neostigmine and Pyridostigmine)

was between 15 to 30 minutes and the effect lasted for about 120 -180 minutes. Their effects are more clearly demonstrated in linear plots of the mean pupil size (mm) versus time (minutes) in Figure 2.

For the pupil dilators, the mean pupil size (± SD, n=4) of TA (Tropicamide and Cycloprntolate) and QA (Hyoscine-N-butyl bromide, Ipratropium, Isopropaminde, and Hexahydroadephenine) was measured at 0, 15, 30, 60, 120 and 180 minutes for different concentrations of each drug (Tables 2 and 3). The onset of the action of the pupil dilatation with all these TAs and QAs lasted between 15-30 minutes and the effect lasted for >180 minutes. Their effects are more clearly shown in linear plots of the mean pupil size (mm) versus time (minutes) in Figures 3.

Table 1. Pupil size (mean and SD) at different concentrations of TA, pupil constrictors.

a.Pilocarpine							
Time (mins)	2%		1%		0.5%		0.25%	
	Mean (mm)	SD	Mean (mm)	SD	Mean (mm)	SD	Mean (mm)	SD
0	4.6	0.3	4.5	0.3	4.5	0.0	4.5	0.3
15	1.6	0.8	2.0	0.4	1.5	0.5	2.5	0.5
30	2.1	0.6	2.0	0.3	1.5	0.6	4.0	0.4
45	2.5	0.6	3.0	0.5	2.0	0.5	4.5	0.3
60	3.4	0.6	3.0	0.5	2.5	0.7	4.5	0.3
120	3.5	0.4	3.5	0.4	3.0	0.5	4.5	0.3
180	3.9	0.3	3.5	0.3	3.0	0.5	4.5	0.3

b.Physostigmine							
Time (mins)	0.5%		0.25%		0.13%		0.06%	
	Mean (mm)	SD	Mean (mm)	SD	Mean (mm)	SD	Mean (mm)	SD
0	4.5	0.1	4.5	0.2	4.8	0.5	4.75	0.5
15	2.25	0.5	2.4	0.8	2.9	0.5	3.4	0.5
30	1.75	0.3	2.1	0.3	2.5	0.4	3.1	0.25
45	2.6	0.3	2.6	0.25	3.1	0.25	3.4	0.25
60	3	0.4	3	0.4	4.4	0.25	4.4	0.25
120	4	0.6	4	0.58	4.8	0.5	4.75	0.5
180	4.5	0.1	4.5	0.1	4.8	0.5	4.75	0.5

c.Neostigmine							
Time (mins)	0.5%		0.25%		0.13%		0.06%	
	Mean (mm)	SD	Mean (mm)	SD	Mean (mm)	SD	Mean (mm)	SD
0	4.48	0.05	4.5	0.02	4.75	0.5	4.95	0.5
15	3.25	0.65	4.25	0.29	4.55	0.5	4.85	0.3
30	3.5	0.91	4.13	0.48	4.65	0.5	4.95	0.5
45	3.75	0.87	3.88	0.63	4.75	0.5	4.95	0.25
60	3.75	0.65	4	0.82	4.75	0.5	4.95	0.5
120	3.95	0.65	4	0.82	4.75	0.5	4.95	0.3
180	4.25	0.65	4.38	0.85	4.75	0.5	4.95	0.5

d.Pyridostigmine							
Time (mins)	0.2%		0.1%		0.05%		0.025%	
	Mean (mm)	SD	Mean (mm)	SD	Mean (mm)	SD	Mean (mm)	SD
0	4.5	0.05	4.8	0.02	4.4	0.5	4.5	0.5
15	2.5	0.65	2.8	0.29	3.4	0.5	4	0.3
30	2	0.91	2.3	0.48	2.5	0.5	3.5	0.5
45	1.5	0.87	2	0.63	2.3	0.5	3.5	0.25
60	1.5	0.65	2	0.82	2.3	0.5	3.5	0.5
120	1.5	0.65	2	0.82	3.2	0.5	4.5	0.3
180	2	0.65	3,2	0.85	4,25	0.5	4.5	0.5

Table 2. Pupil size (mean and SD) at different concentrations of TA, pupil dilators.

a.Tropicamide							
Time (mins)	1%		0.5%		0.25%		0.13%
	Mean (mm)	SD	Mean (mm)	SD	Mean (mm)	SD	Mean (mm)	SD

Time (mins)	Mean (mm)	SD	Mean (mm)	SD	Mean (mm)	SD	Mean (mm)	SD
0	4.5	0	4.5	0.1	4.5	0.3	4.5	0.05
15	7.88	0.25	8.25	0.5	8	0.02	7.63	0.48
30	8.75	0.5	8.25	0.2	7.975	0.05	7.8	0.25
45	8.65	0.5	8.25	0.5	7.95	0.1	7.7	0.1
60	8.75	0.5	8.25	o.3	7.9	0.2	7.7	0.1
120	8.65	0.5	8.1	0.2	7.6	0.1	7.4	0.05
180	8.48	0.47	8	0.5	7.5	0.15	7.2	0.1

b.Cyclopentolate							
Time (mins)	1%		0.5%		0.25%		0.13%	
	Mean (mm)	SD	Mean (mm)	SD	Mean (mm)	SD	Mean (mm)	SD
0	4.5	0.2	4.5	0.1	4.5	0.41	4.38	0.25
15	8.25	0.5	8.05	0.1	8.13	0.25	8.125	0.25
30	8.25	0.5	7.95	0.1	8.13	0.25	8.125	0.25
45	8.5	0.58	7.93	0.15	8	0.41	8	0.41
60	8.5	0.58	8.05	0.1	8	0.41	8	0.41
120	8.4	0.48	7.875	0.25	7.88	0.48	7.63	0.63
180	7.9	0.75	7.5	0	7.5	0.41	7.25	0.29

c.Hyoscine-N-Butyl bromide							
Time (mins)	0.5%		0.25%		0.13%		0.06%	
	Mean (mm)	SD	Mean (mm)	SD	Mean (mm)	SD	Mean (mm)	SD
0	4.5	0.3	4.8	0.5	4.4	0.25	4.75	0.5
15	6.9	0.5	6.8	0.5	6.6	0.5	6.4	0.6
30	7.4	0.75	7.1	0.75	6.9	0.75	6.7	0.6
45	7.6	0.75	7.4	0.75	7.2	0.75	7.1	0.5
60	8.1	0.1	7.9	0.2	7.6	0.2	7.3	0.2
120	8.5	0.6	8.1	0.1	7.9	0.3	7.6	0.3
180	8.7	0.6	8.5	0.05	8.3	0.1	8	0.1

Table 3. Pupil size (mean and SD) at different concentrations of TA, pupil dilators.

d.Ipratropium							
Time (mins)	0.05%		0.025%		0.012%		0.006%	
	Mean (mm)	SD	Mean (mm)	SD	Mean (mm)	SD	Mean (mm)	SD
0	4.5	0.4	4.5	0	4.6	0.25	4.75	0.2
15	5.1	0.5	5.25	0.3	5.4	0.25	5.25	0.5
30	5.9	0.5	5.9	0.5	5.75	0.3	5.375	0.4
45	6.6	0.25	6.5	0.4	6	0.4	5.375	0.2
60	7.1	0.1	7	0	6.125	0.25	5.375	0.6
120	6.9	0.15	7	0	6.125	0.25	5.375	0.5
180	7.0	0.1	7	0	6.125	0.25	5.375	0.3

e.Isopropamide							
Time (mins)	0.5%		0.1%		0.05%		0.025%	
	Mean (mm)	SD	Mean (mm)	SD	Mean (mm)	SD	Mean (mm)	SD
0	4.6	0.2	4.5	0.25	4.8	0	4.5	0.4
15	7.6	0.5	5.5	0.25	5.05	0.3	5.25	0.5
30	8	0.4	6.5	0.3	5.55	0.5	5.5	0.5
45	8	0.2	7.5	0.4	6.8	0.4	5.75	0.25
60	7.8	0.6	7.5	0.25	6.05	0	5.25	0.1
120	7.6	0.5	7	0.25	5.8	0	4.5	0.15
180	6.6	0.3	6.25	0.25	4.8	0	4.5	0.1

f.Hexa-hydro-adiphenine							
Time (mins)	0.2%		0.1%		0.05%		0.025%	
	Mean (mm)	SD	Mean (mm)	SD	Mean (mm)	SD	Mean (mm)	SD
0	4.6	0.2	4.5	0.25	4.6	0	4.7	0.4
15	7.6	0.5	6.8	0.25	5.6	0.3	5.2	0.5
30	8.6	0.4	7.5	0.3	6.6	0.5	5.7	0.5
45	8.8	0.2	7.5	0.4	6.8	0.4	5.7	0.25
60	8.4	0.6	7.5	0.25	6.6	0	5.7	0.1
120	7.5	0.5	6.4	0.25	6.6	0	5.7	0.15
180	6.6	0.3	6.4	0.25	6	0	5.2	0.1

Discussion

The effect of drugs on pupil size after topical administration mainly depends on their diffusion across cornea and conjunctiva [8-9]. TA pupil constrictors and dilators are more lipid-soluble and easily cross cornea/conjunctiva, whereas, QAs poorly cross these tissues. This is why most of the pupil constrictors or dilators available for clinical use are TA in nature [2].

However, in the current study, QA muscarinic agonists (Neostigmine and Pyridostigmine) had the similar onset and duration of action on pupil constriction as TA muscarinic agonists (Pilocarpine and Physostigmine) (Figure 2). Neostigmine, apparently, produces less intense action (maximum pupil constriction of about 2.5 mm), which could be due to its lower concentrations used. One step higher concentration, perhaps, could cause greater pupil constriction equivalent to TA amines (Pilocarpine or Physostigmine).

Similarly, QA muscarinic antagonists (Hyoscine-butyl-bromide, Ipratropium, Isopropamide, and Hexahydro-adiphenine) had the similar effect on pupil size as TA anti-muscarinic drugs (Tropicamide and Cyclopentolate), (Figure 3). However, two QA (Hyoscine-butyl-bromide and Ipratropium) had a slower onset of action. However, another two QA (Isopropamide and Hexahydro-adiphenine) had a faster onset like their TA counterparts (Tropicamide and Cyclopentolate) (Figure 3).

Therefore, the degree of ionization related to the quaternary nature of drugs seems to have little effect on the trans-corneal/conjunctival transport of these drugs and their action on pupil size. The onset and duration of action of these drugs are also not related to their tertiary or quaternary amine nature. For example, pupil dilators Atropine (a TA) and Homatropine (a QA) have a much longer duration of action, 5-6 days, and ½ -1 day, respectively [10]. Moreover, ionization calculated according to the H-H equation had no correlation with their pH-dependent buccal absorption and renal excretion [6-7].

The concept of the relationship between the membrane transport of drugs and their degree of ionization may be true only for some acidic or basic drugs, but not applicable to all acidic or basic drugs [1,4]. This is due to the fact that acidic or basic drugs with the similar pKa and in the same pH of the medium had different capacities to cross buccal mucosa and renal tubular membranes, as mentioned above.

Conclusion

To the best of our knowledge, this was the first experimental study conducted in Saudi Arabia on rabbits to study and compare the effect of topical administration of quaternary amines to tertiary amines on the size of the pupil. Four different

concentrations of each drug were used and rabbits were randomly selected for each concentration of a drug. The pupil size was measured at 0, 15, 30, 60, 120 and 180 minutes after topical administration of the drugs (pupil constrictors and pupil dilators). Pilocarpine and physostigmine from the TA group and neostigmine and pyridostigmine from the QA group were used as - pupil constrictors. Tropicamide and cyclopentolate from TA group and Hyoscine butyl bromide, Ipratropium, Isopropamide, and Hexahydro-adiphenine from QA group were used as pupil dilators.

The present study revealed that the QA muscarinic-agonists demonstrated the similar onset of action and the duration of effect on pupillary constriction when applied topically to the rabbit's eye as their TA counterparts. Similarly, QA muscarinic-antagonists showed similar onset of action and duration of effect on pupil dilatation as their corresponding TA. Therefore, it is concluded that the extent of ionization of QA muscarinic agonists or antagonists had no influence on their diffusion across the ocular tissues cornea and conjunctiva. These drugs can be used topically for pupillary constriction or dilatation.

Study limitations

Differences between rabbit and human eyes could result in altered pharmacokinetics in the rabbit as compared to humans, which is a crucial limitation of this study. Those dissimilarities should be contemplated when deducing the pharmacokinetic properties of the drug, which were obtained from animal studies and applied to humans. Since the current study was experimental in nature, some small differences in environmental conditions and ocular and systemic conditions among the animals used cannot be excluded.

References
1. *Katzung, BG. Introduction. In: Bertram G. Katzung editor. Basic and Clinical Pharmacology. 14th ed. USA: McGraw-Hill Education; 2019.p.9-10.*
2. *Katzung, BG. Cholinoceptor-Activating and Cholinestrase-Inhibiting Drugs. In: Bertram G. Katzung editor. Basic and Clinical Pharmacology. 14th ed. USA: McGraw-Hill Education; 2019. p.115.*
3. *Brunton LL, Hilal-Dandan R, Knollman BC. Muscarinic receptor agonists and antagonists. In: Laurence L. Brunton editor. Goodman and Gillman Pharmacological Basis of Therapeutics. 12th ed. USA: McGraw-Hill Education; 2018. p. 152.*
4. *Kaviani MR. Studies on drug absorption based on form, dosage, drug functional groups and intestinal conditions. Biosci Biotech Res Comm. 2017; Special Issue 2:390-7.*
5. *Rezhdo O, Speciner L, Carrier R. Lipid-associated oral delivery: Mechanisms and analysis of oral absorption enhancement. J Control Release. 2016;240:544-60.*
6. *Lomize AL, Pogozheva ID. Physics-Based Method for Modeling Passive Membrane Permeability and Translocation Pathways of Bioactive Molecules. J Chem Inf Model. 2019;59(7):3198-213.*
7. *Randhawa MA, Iqbal A, Nasimullah M, Akhtar M, Yusuf SM, Turner P. Hendeson-Hasselbalch Equation is inadequate for the measurement of transmembrane diffusion of drugs and buccal drug absorption is a useful alternative. Gen Pharm. 1995; 26(4): 875-9.*
8. *Moiseev RV, Morrison PWJ, Steele F, Khutoryanskiy VV. Penetration enhancers in ocular drug delivery. Pharmaceutics. 2019; 11(7): 321.*
9. *Brunton LL, Hilal-Dandan R, Knollman BC. Ocular Pharmacology. In: Laurence L. Brunton editor. Goodman and Gillman Pharmacological Basis of Therapeutics. 12th ed. USA: McGraw-Hill Education; 2018. p. 1255-9.*
10. *Katzung, BG. Cholinoceptor-Blocking Drugs. In: Bertram G. Katzung editor. Basic and Clinical Pharmacology. 13th ed. USA: McGraw-Hill Education; 2015.p.127.*